

HELMINTHS OF THE SOUTHWESTERN TOAD, *BUFO MICROSCAPHIUS*,  
WOODHOUSE'S TOAD, *BUFO WOODHOUSII* (BUFONIDAE), AND  
THEIR HYBRIDS FROM CENTRAL ARIZONA

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**ABSTRACT.**—The gastrointestinal tracts, lungs, and urinary bladders from 77 *Bufo microscaphus*, 61 *Bufo woodhousii*, and 8 of their hybrids were examined for helminths. One species of trematode (*Glypthelmins quita*), 1 species of cestode (*Distoichometra bufonis*), and 5 species of nematodes (*Aplectana incerta*, *A. itzocanensis*, *Rhabdus americanus*, *Physaloptera* sp., and *Physocephalus* sp.) were found. The greatest prevalence (41%) and mean intensity (231.7) were recorded for *Aplectana incerta* in *Bufo woodhousii*. It appears hybrids harbor fewer parasites than either parent species.

**Key words:** *helminths*, *Bufo microscaphus*, *Bufo woodhousii*, *hybrids*, *Arizona*.

The southwestern toad (*Bufo microscaphus* Cope, 1866) is presently recognized as 3 allopatric subspecies: *B. m. californicus* Camp, 1915, which occurs in coastal southern California and northwest Baja California; *B. m. microscaphus* Cope, 1866, found in southern Nevada and Utah, Arizona, and New Mexico; and *B. m. mexicanus* Brocchi, 1879, which occurs in the Sierra Madre Occidental of central México south to Durango (Price and Sullivan 1988). Woodhouse's toad (*Bufo woodhousii* Girard, 1854) is recognized as 4 subspecies: *B. w. woodhousii* Girard, 1854 occurs in eastern Montana and North Dakota, south through the plains states to central Texas and west of the Rocky Mountains from Idaho south to Colorado and Arizona with isolated populations in west Texas, southeastern California, and along the Oregon-Washington border; *Bufo w. australis* Shannon and Lowe, 1955 is found from central Colorado through New Mexico and Arizona to Sonora, México, and along the Rio Grande drainage into southwest Texas and adjacent México; *Bufo w. velatus* Bragg and Sanders, 1951 is restricted to northeast Texas; and *B. w. fowleri* Hinckley, 1882 is widespread throughout much of the eastern United States south to the Gulf Coast and west to eastern Texas (Behler and King 1979). The toads examined during this study, *B. m. microscaphus* and *B. w. australis*, are known to hybridize in Arizona (Sullivan 1986, Sullivan and Lamb 1988).

Although there are reports of helminths from *B. microscaphus* (Parry and Grundmann 1965) and *B. woodhousii* (Trowbridge and Hefley 1933, Brandt 1936, Walton 1938, Reiber et al. 1940, Kuntz 1941, Kuntz and Self 1944, Rankin 1945, Fantham and Porter 1948, Frandsen and Grundmann 1960, Parry and Grundmann 1965, Campbell 1968, Brooks 1976, Jilek and Wolff 1978, Baker 1985, Hardin and Janovy 1988, McAllister et al. 1989), populations of these toads from Arizona have not been examined. Concern over declining amphibian populations (Heyer et al. 1994) has increased interest in the possible negative effects of parasites on toads. The purpose of this paper is to report on helminths of these toads and their hybrids from Arizona.

This investigation of parasitism in these toads addresses a hypothesis of hybrid zone theory and species boundaries. The hypothesis that populations of hybrid individuals with reduced fitness act as barriers to gene flow between 2 species separated by a hybrid zone (Barton 1979, 1980) could have several mechanisms. One mechanism, increased parasitism of hybrids, is evaluated in this study. Two previous studies of parasitism in vertebrates are split. Hybrid mice (*Mus musculus* × *Mus domesticus*), specifically backcrossed hybrids, had greater numbers of cestode and nematode parasites than either parental species (Sage et al. 1986). Prevalence of monogenean parasites for hybrid minnows (*Barbus barbus* × *Barbus meridionalis*) was

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positively associated with the percentage of *B. meridionalis* genes (Le Brun et al. 1992). If we find that hybrid toads have greater parasitism than each toad species, then parasitism may be a mechanism that reduces hybrid fitness and contributes to the barrier between these 2 toad species.

#### MATERIALS AND METHODS

One hundred forty-six toads were collected in Arizona during 1991–1995; snout-vent length (SVL) was measured to the nearest mm after a minimum of 6 mon in 70% ethanol storage. Toads were identified using a hybrid index (HI) and advertisement call structure, if available. Following Blair (1955), Sullivan (1986), and Sullivan and Lamb (1988), we evaluated the degree of expression of 4 characters to generate the HI score for each toad: dark ventral pigmentation, cranial crest, dorsal stripe, and pale coloration across the eyelids. A numerical score (0, 1, 2, 3) was assigned for each of the following 4 character states: present, weakly present, very weakly present, or absent. A score of 3 was assigned for the presence of dark ventral pigmentation, cranial crests, a dorsal stripe, and absence of a pale bar across the eyelids. This yields scores near 12 ( $4 \times 3$ ) for *B. woodhousii* and 0 ( $4 \times 0$ ) for *B. microscaphus*. Numerous other studies of hybridization between toad species have used a morphological hybrid index such as this (Volpe 1959, Meacham 1962, Henrich 1968, Zweifel 1968). All toads from sites of sympatry with scores of 4 through 8 were considered hybrids, as were all toads with intermediate advertisement calls. Intermediate calls are typical of hybrid toads between these species (Sullivan 1995), and calls have long been used to delimit hybrid toads of other species pairs (Blair 1956, Zweifel 1968, Green 1982). Seventy-seven *Bufo microscaphus* (mean SVL = 61.4 mm  $\pm$  8.7 s, range 34–86 mm, 67 males, 10 females); 61 *Bufo woodhousii* (mean SVL = 74.5 mm  $\pm$  8.8 s, range 49–91 mm, 53 males, 8 females), and 8 hybrids (mean SVL = 60.5 mm  $\pm$  8.4 s, range 45–72 mm, 7 males, 1 female) were examined. Kruskal-Wallis test statistic (45.92, 2 df,  $P < 0.001$ ) indicates significant difference in SVLs for the samples examined. After examination all specimens were deposited in the herpetology collection of Arizona State University (ASU), Tempe. Collection localities

and ASU accession numbers are given in Appendix 1.

Toads were anesthetized by immersion in 1 g/l solution of tricaine methane sulfonate (MS-222, Sigma, St. Louis, MO). Heart, liver, thigh muscle, and kidney were removed and frozen for future genetic analyses. Toads were then fixed in neutral-buffered 10% formalin and moved to ethanol for storage following procedure outlined by Simmons (1987). The body cavity was opened by a longitudinal incision from vent to throat, and the gastrointestinal tract was removed by cutting across the esophagus and rectum. The esophagus, stomach, small intestine, large intestine, lungs, bladder, and coelom were examined under a dissecting microscope. No helminths were found in the esophagus or urinary bladder. All helminths were removed and identified using a glycerol wet mount. Specimens were placed in vials of alcohol and deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705: (accession numbers, Appendix 1).

#### RESULTS AND DISCUSSION

Prevalence, site, and mean intensity for each parasite are given in Table 1. Terminology is in accordance with Margolis et al. (1982). One species of trematode (*Glypthelminis quieta* [Stafford, 1900]), 1 species of cestode (*Distiochometra bufonis* Dickey, 1921), and 5 species of nematodes (*Aplectana incerta* Caballero, 1949, *Aplectana itzocanensis* Bravo Hollis, 1943, *Rhabdias americanus* Baker, 1978, *Physaloptera* sp. [larvae only], and *Physocephalus* sp. [larvae only]) were found. It would appear from Table 1 that both species and their hybrids are susceptible to infection by the same parasites. The greatest prevalence (41%) and mean intensity (231.7) in our study were recorded for *Aplectana incerta* in *Bufo woodhousii*. Thirty-four of 77 (44%) *Bufo microscaphus* (30/67, 45% males; 4/10, 40% females), 51 of 61 (84%) *B. woodhousii* (45/53, 85% males; 6/8, 75% females), and 4 of 8 (50%) hybrids (3/7 males, 1/1 female) were infected. Males and females of both *Bufo microscaphus* ( $\chi^2 = 1.17$ , 1 df,  $P > 0.05$ ) and *B. woodhousii* ( $\chi^2 = 2.79$ , 1 df,  $P > 0.05$ ) did not differ significantly in helminth prevalence. There were too few female hybrid toads for chi-square analysis. There was statistical difference in abundance of nematodes between *B. microscaphus* and *B. woodhousii* ( $\chi^2 = 23.72$ ,

TABLE 1. Prevalence, mean intensity (range), and location of helminths from *Bufo microscaphus*, *B. woodhousii*, and their hybrids from Arizona.

Parasite species	<i>Bufo microscaphus</i> (N = 77)			<i>Bufo woodhousii</i> (N = 61)			Hybrids (N = 8)		
	Prevalence (%)	Intensity (range)	Location	Prevalence (%)	Intensity (range)	Location	Prevalence (%)	Intensity (range)	Location
TREMATODA									
<i>Glythelminis quieta</i>	1	1.0	b	2	2.0	b	13	1.0	b
CESTODA									
<i>Distoichometra bufonis</i>	14	2.9 (1-6)	b	35	2.0 (1-5)	b	13	1.0	b
NEMATODA									
<i>Aplectana incerta</i>	1	156.0	b,c	41	231.7 (23-564)	b,c	—	—	—
<i>Aplectana itzocanensis</i>	19	75.0 (1-373)	b,c	26	43.2 (1-204)	b,c	25	1.0	c
<i>Physaloptera</i> sp. (larva)	16	5.5 (1-31)	a	5	6.0 (2-11)	a	13	1.0	a
<i>Physocephalus</i> sp. (larvae)	1	104.0	d	—	—	—	—	—	—
<i>Rhabdias americanus</i>	5	2.0 (1-3)	e	35	21.7 (1-111)	e	—	—	—

a = stomach, b = small intestines, c = large intestines, d = cysts on stomach wall, e = lungs.

1 df,  $P < 0.001$ ). When the intermediate prevalence (50%) of the small hybrid sample ( $N = 8$ ) was included in the chi-square calculation, statistical significant difference remained ( $\chi^2 = 23.97$ , 2 df,  $P < 0.001$ ).

To test for difference in infection rate, we used a Kruskal-Wallis rank-order statistic because of the great variation in mean intensity of parasites harbored by *B. microscaphus*, *B. woodhousii*, and their hybrids (116.3, 19.4, 1.3, respectively) and the relatively small sample of hybrids ( $N = 8$ ). This test revealed that hybrid individuals had fewer parasites than do individuals of either species. Examination of more hybrids could strengthen this result. Subsequent work to determine the importance of age, genetic factors, nutrition, and ecology would also help to establish the significance of hybrid ancestry on parasite levels.

Infected frogs appeared healthy; i.e., none were emaciated and there were no gross abnormalities. Thus, the presence of helminths did not appear to adversely affect the populations of *B. microscaphus*, *B. woodhousii*, or their hybrids. In a study on Couch's spadefoot (*Scaphiopus couchii*) from Arizona, Tinsley (1990) found no correlation between presence of the trematode *Pseudodiploorchis americanus* and mating success, although the presence of *P. americanus* reduced fat reserves during hibernation.

*Bufo microscaphus* is a new host record for *Distoichometra bufonis*, *Aplectana incerta*, *A. itzocanensis*, *Physocephalus* sp., and *Rhabdias americanus*. *Bufo woodhousii* is a new host

record for *Aplectana incerta* and *Physaloptera* sp. *Bufo w. woodhousii* has been reported by Baker (1985) to host *Aplectana itzocanensis*. *Bufo microscaphus*  $\times$  *B. woodhousii* hybrid is a new host record for *Distoichometra bufonis*, *Glythelminis quieta*, *Aplectana itzocanensis*, and *Physaloptera* sp. *Glythelminis quieta* in Arizona is a new locality record.

With the exception of *Glythelminis quieta*, all helminths found in our study have been previously reported in other desert toads from Arizona (Table 2). *Glythelminis quieta* has previously been reported in *Bufo microscaphus* from Utah (Parry and Grundmann 1965) and in *Bufo woodhousii* from Nebraska (Brooks 1976). It is widely distributed in anurans in North America (Prudhoe and Bray 1982). The distribution of *Distoichometra bufonis*, *Aplectana incerta*, *A. itzocanensis*, *Physaloptera* sp., and *Rhabdias americanus* in North American toads has previously been discussed (Goldberg and Bursey 1991a, Goldberg et al. 1996). *Aplectana incerta*, *A. itzocanensis*, and *Rhabdias americanus* have direct life cycles; *Distoichometra bufonis*, *Physaloptera* sp., and *Physocephalus* sp. have indirect life cycles and require at least 1 intermediate host (Anderson 1992, Smyth 1994). Because these helminths are not species specific and occur in a variety of amphibians, the distribution of intermediate hosts may play an important role in determining the distribution of those parasites with indirect life cycles. The conditions responsible for determining distribution of the parasites with direct life cycles have yet to be defined.

TABLE 2. Helminth community of desert toads from Arizona.

Helminth	Host	Reference
TREMATODA		
<i>Glypthelmins quieta</i>	<i>Bufo microscaphus</i>	This study
	<i>B. woodhousii</i>	This study
<i>Pseudodiploorchis americanus</i>	<i>Scaphiopus couchii</i>	Tinsley 1990
CESTODA		
<i>Distoichometra bufonis</i>	<i>Bufo cognatus</i>	Goldberg and Bursey 1991a
	<i>B. microscaphus</i>	This study
	<i>B. punctatus</i>	Goldberg and Bursey 1991b
	<i>B. retiformis</i>	Goldberg et al. 1996
	<i>B. woodhousii</i>	This study
	<i>Scaphiopus couchii</i>	Goldberg and Bursey 1991a
<i>Nematotaenia dispar</i>	<i>Bufo alvarius</i>	Goldberg and Bursey 1991a
NEMATODA		
<i>Aplectana incerta</i>	<i>Bufo microscaphus</i>	This study
	<i>B. retiformis</i>	Goldberg et al. 1996
	<i>B. woodhousii</i>	This study
	<i>Scaphiopus couchii</i>	Goldberg and Bursey 1991a
<i>Aplectana itzocanensis</i>	<i>Bufo alvarius</i>	Goldberg and Bursey 1991a
	<i>B. cognatus</i>	Goldberg and Bursey 1991a
	<i>B. microscaphus</i>	This study
	<i>B. punctatus</i>	Goldberg and Bursey 1991b
	<i>B. retiformis</i>	Goldberg et al. 1996
	<i>B. woodhousii</i>	This study
<i>Oswaldocruzia pipiens</i>	<i>Bufo alvarius</i>	Goldberg and Bursey 1991a
	<i>B. cognatus</i>	Goldberg and Bursey 1991a
	<i>B. punctatus</i>	Goldberg and Bursey 1991b
	<i>B. retiformis</i>	Goldberg et al. 1996
	<i>Scaphiopus couchii</i>	Goldberg and Bursey 1991a
<i>Physaloptera</i> sp. (larva)	<i>Bufo alvarius</i>	Goldberg and Bursey 1991a
	<i>B. cognatus</i>	Goldberg and Bursey 1991a
	<i>B. microscaphus</i>	This study
	<i>B. retiformis</i>	Goldberg et al. 1996
	<i>B. woodhousii</i>	This study
<i>Physocephalus</i> sp. (larva)	<i>Bufo alvarius</i>	Goldberg and Bursey 1991a
	<i>B. microscaphus</i>	This study
	<i>B. retiformis</i>	Goldberg et al. 1996
<i>Rhabdias americanus</i>	<i>Bufo alvarius</i>	Goldberg and Bursey 1991a
	<i>B. cognatus</i>	Goldberg and Bursey 1991a
	<i>B. microscaphus</i>	This study
	<i>B. retiformis</i>	Goldberg et al. 1996
	<i>B. woodhousii</i>	This study

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## APPENDIX 1

Localities and museum (ASU) numbers for specimens examined:

***Bufo microscaphus*:** Maricopa County ( $N = 6$ ) (34°00'N, 112°45'W, elev 603 m) ASU 30360-61, 30369-72; Yavapai County ( $N = 61$ ); 7 from (34°24'N, 112°13'W, elev. 1323 m) ASU 30328-31, 30347-49; 6 from (34°06'N, 112°09'W, elev 603 m) (ASU 29166-67, 29170-71, 30351, 30375); 4 from (34°04'N, 112°09'W, elev 488 m) (ASU 30377, 30379-81); 34 from (34°05'N, 112°07'W, elev 616 m) ASU 28845-50; 28852-57, 29172-83, 30334-40; 30386-88; 10 from (34°24'N, 112°08'W, elev. 1140 m) ASU 30487-96; Coconino County ( $N = 10$ ) (34°24'N, 112°08'W, elev 2094 m) ASU 30477-86.

***Bufo woodhousii*:** Maricopa County ( $N = 53$ ); 14 from (33°38'N, 112°28'W, elev 410 m) ASU 28821-27, 28829-

31, 30356-59; 19 from (33°56'N, 112°08'W, elev 628 m) ASU 28818-19, 28828, 28835, 30362-64, 30366-68, 29151-59; 2 from (33°36'N, 112°15'W, elev. 365 m) ASU 28834, 28836; 7 from (33°39'N, 112°14'W, elev. 389 m) ASU 30497-503; 11 from (33°36'N, 112°11'W, elev. 372 m) ASU 30504-14; Yavapai County ( $N = 8$ ); 7 from (34°06'N, 112°09'W, elev 488 m) (ASU 29165, 29167-69; 30345, 30350, 30355, 30376); 1 from (34°04'N, 112°09'W, elev 488 m) (ASU 30385).

**Hybrids:** Yavapai County ( $N = 8$ ); 7 from (34°06'N, 112°09'W, elev 603 m) ASU 30346, 30352-54, 30373-74, 30382; 1 from (34°04'N, 112°09'W, elev 488 m) ASU 30378.

Accession numbers for helminths in the U.S. National Parasite Collection (USNPC):

***Bufo microscaphus*:** *Distoichometra bufonis* (S5910); *Glypthelminis quieta* (S5921); *Aplectana incerta* (S5911); *Aplectana itzacanensis* (S5912); Physalopteridae (S5915); *Physocephalus* sp. (S5914); *Rhabdias americanus* (S5913). ***Bufo woodhousii*:** *Distoichometra bufonis* (S5916); *Glypthelminis quieta* (S5921); *Aplectana incerta* (S5917); *Aplectana itzacanensis* (S5918); Physalopteridae (S5920); *Rhabdias americanus* (S5919). **Hybrids:** *Distoichometra bufonis* (S5922); *Glypthelminis quieta* (S5921); *Aplectana itzacanensis* (S5923); Physalopteridae (S5924).